A morphometric and anatomical study of the *Darwinia diosmoides* complex (Myrtaceae) in south-western Australia

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Abstract

Rye, B. L. A morphometric and anatomical study of the *Darwinia diosmoides* complex (Myrtaceae) in south-western Australia. Nuytsia 4 (3): 411-421 (1983). Both floral morphology and leaf anatomy proved to be of little value in distinguishing the three variants of the *Darwinia diosmoides* complex. Morphometric analysis of foliar characters confirmed that the northern variant, which differs in chromosome number and several other respects, should be recognized as a new species (*D. capitellata* Rye). However, the two southern variants showed complete intergradation in their foliar characters; hence they were not considered sufficiently distinct to be given formal taxonomic rank.

Introduction

The variants and close relatives of *Darwinia diosmoides* (DC.) Benth., which will be referred to collectively as the *D. diosmoides* complex, are distributed over a wide area in the southwest of Western Australia. The first name to have been applied to members of the complex was *Genetyllis diosmoides* DC., which Bentham (1865) transferred to *Darwinia*. Turczaninow (1847) described two short-leaved variants as *Genetyllis affinis* and *G. drummondii* (misspelt *drumondii*) respectively, claiming that the former had a smooth calyx tube. However, Bentham (1865, 1867), who had rightly noted that the calyx tube of *G. affinis* had protuberances similar to those of *Darwinia diosmoides*, reduced both this and *G. drummondii* to synonyms of *D. diosmoides*.

All variants of the *Darwinia diosmoides* complex are bushy shrubs, up to one metre high, bearing numerous head-like condensed racemes of tiny white flowers during the spring and summer months. Three main morphological variants may be recognized in the complex, occupying distinct habitats and geographical areas. However, the first two appear to show considerable intergradation, particularly in the east of their ranges. Figure 1 illustrates the distributions of these variants, which are characterized as follows:

(1) South-coastal, associated with cliffs and rock outcrops, n=7, 14. Includes the type specimen of *Darwinia diosmoides*.
(2) Central, associated with salt lakes, n = 14. Includes the type specimen of *Genetyllis affinis* and probably *G. drummondii*.
(3) Northern, on sandplain areas and breakaways, n = 12. Now described as *Darwinia capitellata* Rye (Rye 1983).

The *Darwinia diosmoides* complex was chosen for a special study because it had been found earlier (Rye 1979) to show both dysploid and polyploid variation in chromosome number, having n = 7, 12 and 14 as indicated above. Smith-White (1954) reported a further chromosome number, n = 6, from material collected at Albany but there is no voucher specimen for the record. Presumably, the n = 6 record was made from a different species because two Albany populations sampled by Rye (1979) each had n = 14.

Figure 1. Distribution of the *Darwinia diosmoides* complex.

△, A-D: populations of the northern variant (*Darwinia capitellata*); ▽, E-X: central and south-coastal populations (*D. diosmoides*). Dotted lines indicate the overall ranges of the two species.

Aside from its unique chromosome number (n = 12), habitat and geographical distribution, the northern variant (*Darwinia capitellata*) can be distinguished from the remainder of the complex by its vegetative characteristics, inflorescence structure and distribution of oil glands. However, it does not show any obvious differences in floral morphology from the other variants. Populations of the central variant tend to have tiny appressed leaves, whereas the south-coastal variant tends to have longer, more spreading leaves, but again there are no obvious differences in floral morphology. It was not certain whether there was sufficient discontinuity between the central and south-coastal variants, especially in the eastern part of their ranges, to warrant their formal recognition as distinct taxa.

The aim of this study was to investigate the taxonomy of the three variants by means of morphometric analyses and examination of leaf anatomy, in particular to determine whether:

(1) the northern variant could be distinguished from the remainder of the complex by its floral morphology.
(2) the two southern variants were sufficiently distinct to be given formal taxonomic rank.

Materials and Methods

Canonical Variate Analysis

Morphometric data were collected in a form appropriate for the application of the canonical variate analysis employed in Phillips et al. (1973), which should be consulted for details of the mathematical basis of this technique. The analysis is de-

signed to give the greatest possible separation of a number of groups, comprising numerous representatives, each measured for a number of variables. In this case the groups were plant populations, represented by numerous plant individuals, and the variables measured were the 9 floral and 6 foliar characters illustrated in Figure 2. Maximum separation can be achieved by a multidimensional representation of the groups but the first two axes account for the bulk of the separation. These two dimensions can be readily illustrated in the form of a scatter diagram. For each axis the individuals of each group are assigned a 'canonical variate score' which consists of a combination of all the measured variables, each given a different weighting according to its usefulness in achieving the separation. A quantitative measure of the overall degree of separation achieved by the analysis can be obtained by calculating the 'canonical root', which is higher in value the greater the separation.

The validity of the canonical variate scores for distinguishing the groups can be tested by sampling extra individuals, referred to here as 'testers', including some from the populations that constitute the groups. If the separation is soundly based, the canonical scores of the testers should place them close to other members of their groups so that they can be readily identified.

Table 1. Details of populations sampled for the canonical variate analyses of the *Darwinia diosmoides* complex.

Population			No. of individuals	
*Symbol	Locality	Voucher	Group typifiers	Testers
Northern variant *(D. capitellata)*				
A	Paynes Find	B. L. Powell 73012	20	5
B	Perenjori (H)	C. A. Gardner s.n.	—	1
C	Mount Magnet (H)	B. L. Powell 74045	—	1
D	Coolcalalaya (H)	J. S. Beard 7148	—	1
Central variant				
E	Damboring	B. L. Rye 77025	20	5
F	Quairading	B. L. Powell 74126	25	6
G	Cultivated, ex Cunderdin	—	—	2
H	Cultivated Kings Park	—	—	1
I	Pingaring	B. L. Rye 77023	—	1
J	Lake King (H)	A. S. George s.n.	—	1
K	One Mile Rocks (H)	A. S. George 10471	—	1
Intermediate between central and south coastal variants				
L	Stirling Range	—	—	1
M	Hamersley River (H)	A. S. George 7091	—	1
N	Twilight Cove (H)	E. C. Nelson 17172	—	1
O	Cape Arid (H)	R. D. Royce 9886	—	1
P	Dalyup-Esperance (H)	T. E. H. Aplin 2646	—	1
Q	Bedford Harbour (H)	J. S. Beard 2270	—	1
R	Mount Short (H)	E. Wittwer 1883	—	1
South-coastal variant				
S	Duke of Orleans Bay (H)	R. D. Royce 6234	—	1
T	East Mt Barren (H)	C. A. Gardner & W. E. Blackall	—	1
U	Mid Mt Barren (H)	C. A. Gardner 9221	—	1
V	Cultivated, ex Pt Ann	—	—	2
W	Two Peoples Bay	B. L. Powell 74130	30	6
X	Albany	B. L. Powell 74131	10	4

* Reference letter for Figures 1, 3 and 4.
(H) Sampled from herbarium specimen in PERTH.

In this study two separate analyses were undertaken, using floral and foliar characters respectively. Details of the populations sampled are given in Table 1. Five populations were selected as the groups for the initial separation and about five individuals from each were reserved for use as testers. Six additional plants, most of them cultivated in Perth, were used as testers for both the floral and foliar analyses. In order to provide testers from throughout the range of the *Darwinia diosmoides* complex, portions of fifteen herbarium specimens were given a prolonged soaking in a detergent solution. These specimens were used only for the foliar analysis. The five main populations included one population of the northern variant of the complex, two typical of the central variant and two typical of the south-coastal variant, while the tester populations encompassed the three variants and also a number of populations that appeared to be intermediate in morphology between the typical central and south-coastal variants.

Anatomy

Leaves collected from the following populations were embedded in wax or GMA resin, cut into 2μm or 6μm sections respectively, then stained in toluidine blue or saffranin/fast green:

Northern variant—Paynes Find (location A in Figure 1)

Central variant—Damboring (E)
—Cultivated ex Cunderdin (G)
—Cultivated in Kings Park (H)

South-coastal variant—Cultivated ex Pt Ann (V)

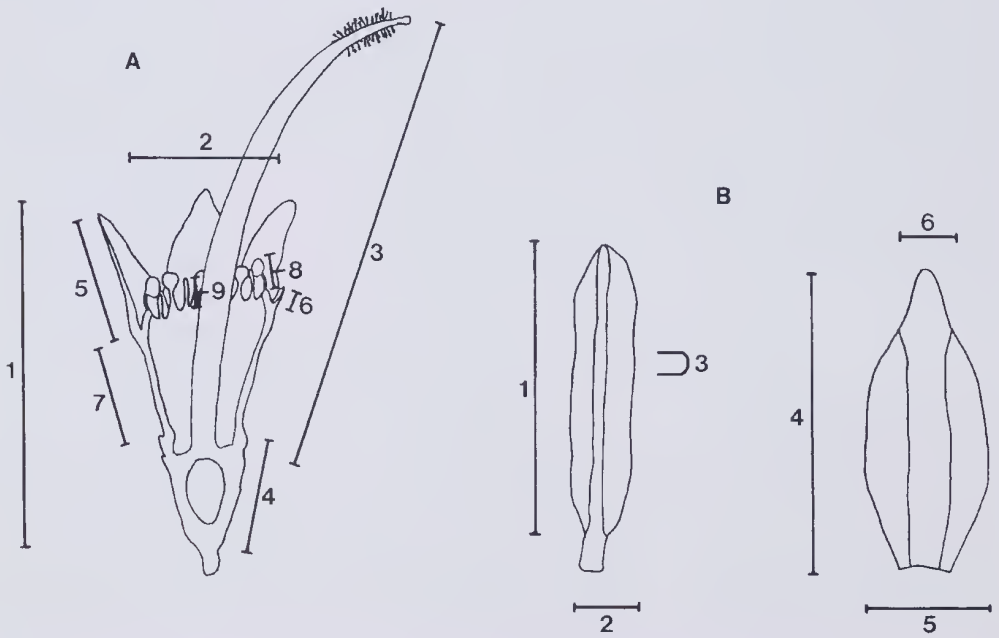

Figure 2. Characters measured for the canonical variate analyses of the *Darwinia diosmoides* complex.

A. Floral characters: 1 — Flower length. 2 — Flower width. 3 — Style length. 4 — Ovary length. 5 — Petal length. 6 — Sepal length. 7 — Floral tube length. 8 — Stamen length. 9 — Staminode length.

B. Foliar characters: 1 — Leaf length. 2 — Leaf width. 3 — Leaf thickness. 4 — Bracteole length. 5 — Bracteole width. 6 — Bracteole midrib width.

Table 2. Mean measurements for populations used in the canonical variate analyses of the *Darwinia diosmoides* complex.

Characters measured	Population means (mm)				
	Paynes Find	Damboring	Quairading	Two Peoples Bay	Albany
Floral analysis					
Flower length	4.27 (±0.07)	4.26 (±0.06)	4.26 (±0.06)	3.85 (±0.05)	4.26 (±0.09)
Flower width	1.82 (±0.02)	1.75 (±0.03)	1.82 (±0.03)	1.67 (±0.03)	1.82 (±0.04)
Style length	6.01 (±0.10)	5.47 (±0.08)	4.60 (±0.07)	4.89 (±0.07)	5.10 (±0.11)
Ovary length	1.54 (±0.03)	1.78 (±0.04)	1.63 (±0.04)	1.43 (±0.03)	1.65 (±0.06)
Petal length	2.10 (±0.04)	1.99 (±0.03)	1.84 (±0.04)	1.54 (±0.03)	1.64 (±0.04)
Sepal length	0.61 (±0.02)	0.46 (±0.02)	0.39 (±0.02)	0.34 (±0.01)	0.27 (±0.01)
Upper floral tube length	0.98 (±0.02)	0.99 (±0.02)	0.79 (±0.02)	0.87 (±0.02)	0.89 (±0.02)
Stamen length	0.75 (±0.02)	0.65 (±0.01)	0.50 (±0.01)	0.41 (±0.01)	0.50 (±0.01)
Staminode length	0.35 (±0.01)	0.39 (±0.01)	0.38 (±0.01)	0.40 (±0.01)	0.38 (±0.02)
Foliar analysis					
Leaf length	4.23 (±0.16)	1.96 (±0.06)	2.63 (±0.05)	3.88 (±0.10)	4.29 (±0.23)
Leaf width	1.02 (±0.02)	1.08 (±0.03)	1.04 (±0.04)	0.53 (±0.01)	0.63 (±0.03)
Leaf thickness	0.66 (±0.01)	0.71 (±0.02)	0.60 (±0.02)	0.48 (±0.01)	0.56 (±0.02)
Bracteole length	2.33 (±0.07)	2.49 (±0.06)	3.03 (±0.06)	2.65 (±0.05)	3.10 (±0.09)
Bracteole width	1.47 (±0.05)	1.01 (±0.02)	1.00 (±0.03)	1.00 (±0.02)	1.05 (±0.03)
Bracteole midrib width	0.14 (±0.01)	0.50 (±0.02)	0.54 (±0.03)	0.68 (±0.02)	0.72 (±0.03)

Results

Morphometrics

Table 2 lists the group means for each of the characters measured in the floral and foliar canonical analyses. The character weightings (standardized character coefficients) and canonical roots for the first two canonical variates are given in Table 3 and scatter diagrams showing the positions of all group and tester individuals with respect to the first two canonical axes are given in Figures 3 and 4.

In the analysis based on foliar characters, the bracteole midrib width and bracteole total width (these were negatively correlated) contributed most to the first canonical variate, which accounted for 57% of the total group separation. Leaf length and

Table 3. Character weightings and canonical roots obtained in the canonical analyses of the *Darwinia diosmoides* complex.

Character weightings and measures of separation	Canonical variate 1	Canonical variate 2
Floral analysis		
Character coefficients (standardized)		
Flower length	—0.228	0.332
Flower width	—0.071	0.288
Style length	0.325	—0.476
Ovary length	0.008	0.371
Petal length	0.460	0.407
Sepal length	0.218	—0.201
Floral tube length	0.224	—0.553
Stamen length	0.778	0.149
Staminode length	—0.437	—0.474
Canonical root	7.18	1.32
% Total separation	77.1	14.2
Foliar analysis		
Character coefficients (standardized)		
Leaf length	—0.155	0.970
Leaf width	0.350	—0.568
Leaf thickness	0.201	—0.068
Bracteole length	—0.097	—0.829
Bracteole width	0.757	0.465
Bracteole midrib width	—1.000	0.014
Canonical root	13.01	9.15
% Total separation	56.8	39.9

bracteole length were positively correlated and contributed most to the second canonical variate, which accounted for a further 40% of the total separation. Thus only 3% of the total group separation was not represented in Figure 3. The canonical roots were relatively high (13 and 9) and the 5 groups (populations) separated into 3 very distinct entities corresponding with the 3 variants of the *Darwinia diosmoides* complex. Testers from the same five populations invariably could be identified according to their variant of the complex, although not necessarily to their particular population; this demonstrated that the separation of the groups into three entities was valid.

The other testers in the foliar analysis showed a greater deviation from the various group means. Testers derived from populations of the northern variant *Darwinia capitellata* could all be readily identified as this variant, supporting its recognition as a distinct taxon. However, many of the testers derived from populations of the central and south-coastal variants fell into the region separating the groups that represented these two variants. When all these populations were considered jointly there was no obvious region of discontinuity which could permit the central and south-coastal populations to be separated into distinct taxa.

In the analysis of floral characters, very little separation of the groups was achieved as can be seen from Figure 4 and the low values of the canonical roots (7 and 1). The three floral characters contributing most to the separation (77% of the total separation) achieved by the first canonical variate were stamen length, petal length and staminode length. There was a north-south trend in each of these charac-

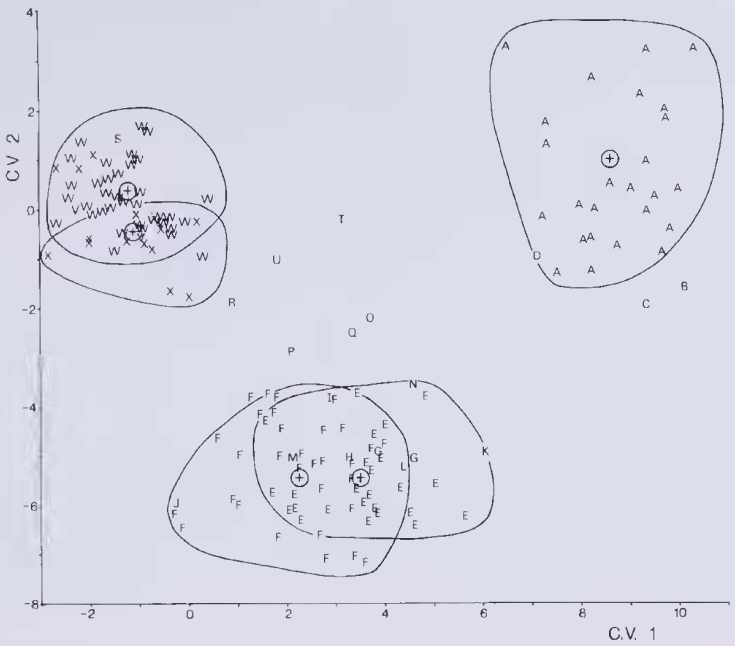

Figure 3. Separation achieved by the first two canonical variates in the analysis of foliar characters of the *Darwinia diosmoides* complex.

A-X: the populations represented by these letters are indicated in Table 1. Lines enclose the members of each group (A, E, F, X and W) but not necessarily the testers of those groups. C.V. — canonical variate; ⊕—population mean.

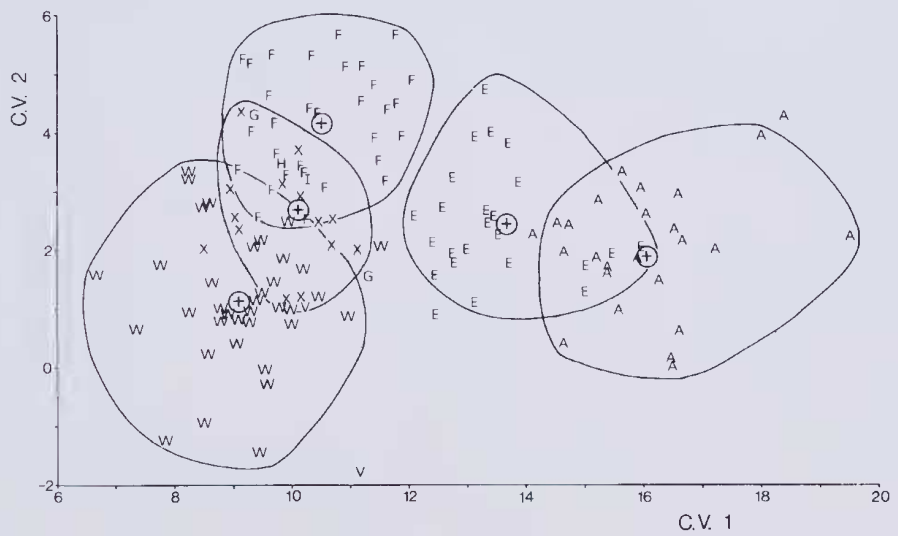

Figure 4. Separation achieved by the first two canonical variates in the analysis of floral characters of the *Darwinia diosmoides* complex.

A-X: the populations represented by these letters are indicated in Table 1. Lines enclose the members of each group (A, E, F, X and W) but not necessarily the testers of these groups. C.V.—canonical variate; ⊕—population mean.

ters, the northern populations tending to have the longer stamens, longer petals and shorter staminodia. The difference between the lengths of the stamens and staminodia provided the most useful floral characteristic for distinguishing different populations. In young flowers of the most northerly populations, the staminodia appeared distinctly shorter than the stamens whereas they appeared equally long in the southernmost populations. However, all the floral characters exhibited continuous variation and tester individuals could not be reliably identified by their canonical variate scores. Consequently the floral analysis provided evidence for the retention of all variants of the *Darwinia diosmoides* complex as a single species.

In summary, the northern variant (*Darwinia capitellata*) could be identified by its foliar characters, chiefly its large midrib width/bracteole width ratio, but not by its floral characters. The two southern variants (*D. diosmoides*) could not be reliably distinguished from one another either by foliar or floral characters.

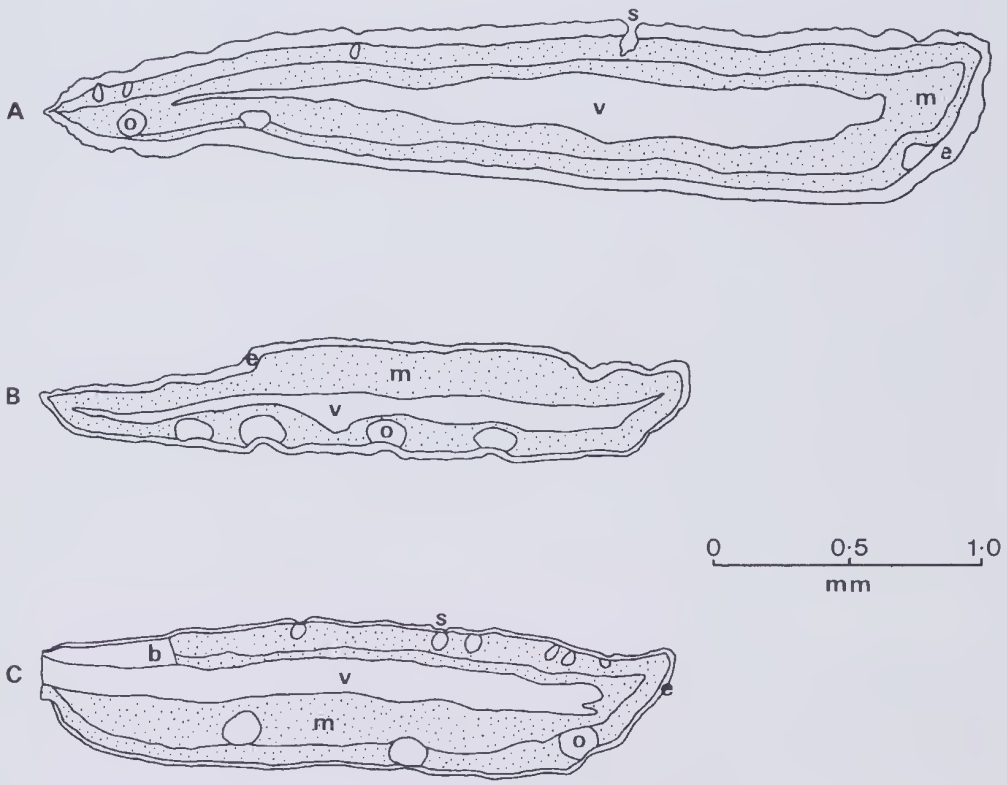

Figure 5. Leaf anatomy in the *Darwinia diosmoides* complex. (Longitudinal sections with the adaxial surface uppermost, the apex to the right.)

A—Northern variant (*Darwinia capitellata*) from Paynes Find. B — Central variant from Damboring. C — Cultivated at Kings Park.

b — bulliform cells (subepidermal); e — epidermis and cuticle; m — mesophyll (dotted), the outer layer palisade parenchyma, the inner layer spongy parenchyma; o — oil glands and associated cells; s — stomata and stomatal cavities; v — vascular tissue of the midrib and associated fibres.

Anatomy

The leaves of the northern variant had thicker cuticles than those of the two southern variants but showed no other notable anatomical differences (see Figure 5). The only qualitative difference in leaf anatomy found between the populations sampled was the presence/absence of large thin-walled cells (apparently bulliform cells) in a subepidermal adaxial region above the midrib (illustrated in Figure 5c). These cells were present only in a cultivated plant (n = 14) of unknown origin, which appeared to have been derived from a population of the central variant. This characteristic did not assist in distinguishing the variants in the *Darwinia diosmoides* complex because it was not present in two other populations of the central variant.

Discussion

The canonical variate analysis provides an objective means of assessing the relative values of various quantitative characters for taxonomic divisions. While its main function is to maximize the separation of predetermined groups and to indicate which characters are most useful in achieving this separation, it also serves to indicate which groups should be combined on the basis of the characters measured.

In the present example, the analyses demonstrated that the five *Darwinia* groups showed continuous variation in their floral characters. They also showed that two pairs of groups could not be distinguished by their foliar characters. The groups belonging to each of these pairs were populations of the same variant of the *Darwinia diosmoides* complex. If they had been combined so that each variant was represented by only one group (i.e. three groups used for the analysis instead of five), the analysis could have given these entities greater cohesion and separated them more successfully from one another. The locations of the tester populations in Figure 3 confirmed the distinctiveness of the northern variant but demonstrated that the two southern variants of the *D. diosmoides* complex could not be adequately distinguished.

The use of herbarium specimens for many of the testers probably introduced a small error into the measurements because the effects of dehydration and pressing on the size and shape of the leaves may not have been completely rectified by rehydration. A more significant source of variability in the measurements was environmental. Considerable seasonal and yearly variation in leaf size was observed in natural populations. By increasing the intra-populational variability, this effect would have tended to obscure the differences between populations since the plants used in this study, particularly the herbarium specimens, were sampled in varied season over many years.

If the observed morphological differences between populations occupying different habitats were largely due to environmental effects rather than genetic factors, they would not be valid criteria for taxonomic divisions. However, specimens from widely separated natural populations maintained large morphological differences when grown under uniform conditions in Perth gardens. Several cultivated plants of known origin were tested in the foliar analysis and all were correctly identified to their variant of the *Darwinia diosmoides* complex.

Floral characters showed less intra-populational variability than foliar characters (see Table 3) and also appeared to show less variability from year to year. They would, therefore, have been more suitable criteria than the foliar characters for taxonomic divisions if they had shown more significant differences between populations.

The anatomical differences between the three variants of the *Darwinia diosmoides* complex have only been examined briefly in the present study. The observed variation in cuticle thickness was correlated with, and presumably a function of, the aridity of the habitat from which the leaf samples were taken. The thinnest cuticles occurred in the well watered cultivated plants. In view of the observed differences in leaf anatomy between populations of the central variant, it is evident that many more populations would need to be surveyed before any firm conclusions could be reached regarding the taxonomic value of anatomical critera. Possibly studies of flower or stem anatomy would prove more valuable than leaf anatomy for distinguishing the taxa.

It is concluded that the two southern variants are not sufficiently distinct in any of the characters examined here to be formally recognized as intraspecific taxa of *Darwinia diosmoides*. The northern variant shows no significant difference in either its floral morphology or leaf anatomy from the two southern variants but differs in its foliar characters and in several other morphological characters. Details of the latter characters are given in the accompanying paper (Rye 1983), in which the northern variant is described as the new species, *D. capitellata* Rye.

The *Darwinia diosmoides* complex (with n = 7, 12, 14) and its close relatives, *D. vestita* (n = 9) and *Actinodium cunninghamii* (n = 6), are notable among the Myrtaceae for their variety of chromosome numbers. The ancestral chromosome number among these taxa is evidently n = 9, dysploid reduction having given rise to the n = 7 and n = 6 cytotypes (Smith-White 1959, Rye 1979). This suggests that *D. capitellata* is derived from *D. diosmoides* or a *diosmoides*-like ancestor, not vice versa. The tetraploid n = 12 cytotype may have been derived either by a reduction at the tetraploid level (14 → 12) or by polyploidy from a hypothetical n = 6 ancestor, which in turn had been derived by dysploid reduction from n = 7 (7 → 6⇒ 12).

Although the exact origin of the new chromosome number in *Darwinia capitellata* is not known, the establishment of the new number may have been instrumental in achieving sufficient reproductive isolation to permit the morphological divergence of the species from *D. diosmoides*. The two species are allopatric, precluding natural hybridization. It would be worthwhile conducting artifical hybridization studies on cultivated plants to determine the degree and nature of reproductive isolation when the spatial barrier is removed.

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